



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

050801

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

Tempo[®] EB (*Enterobacteriaceae*)

manufactured by

bioMérieux, sa

Chemin de l'Orme

69208 Marcy l'Etoile, France

This method has been evaluated in the AOAC[®] Performance Tested MethodsSM Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Performance TestedSM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 07, 2017 – December 31, 2018). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

December 07, 2017

Date

METHOD AUTHORS

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SUBMITTING COMPANYbioMérieux, Inc.
595 Anglum Drive
Hazelwood, MO 63042 USA**KIT NAME(S)**Tempo® EB (*Enterobacteriaceae*)**CATALOG NUMBERS**

80003

INDEPENDENT LABORATORYQ Laboratories, Inc
1400 Harrison Avenue
Cincinnati, OH 45214
USA**AOAC EXPERTS AND PEER REVIEWERS**Thomas Hammack¹, Michael Brodsky², Greg Siragusa³
¹ US Food and Drug Administration, Center for Food Safety and Applied
Nutrition, College Park, MD, USA
² Brodsky Consultants, Thronhill, Ontario, CANADA
³ Agtech, USA**APPLICABILITY OF METHOD**Target organism – *Enterobacteriaceae*Matrices – (25 g) - raw ground pork, fresh ground beef, heat processed
cooked roast beef, ground chicken, frozen cooked chicken, heat processed
grilled chicken, frozen catfish, heat processed frozen fish, raw cod, bagged
salad, frozen green beans, has brown potatoes, rice, vanilla ice cream,
pasteurized milk, milk powder, pasteurized eggs, dry pet foodPerformance claims - TEMPO EB (*Enterobacteriaceae*) is an automated test
for use with TEMPO, for the enumeration of enterobacteria in 22-27 hours
in food products.**REFERENCE METHOD**Compendium of Methods for the Microbiological Examination of Foods,
(2001) 4th Edition, Chapter 8: *Enterobacteriaceae*, Coliforms, and
Escherichia coli as Quality and Safety Indicators (2)**ORIGINAL CERTIFICATION DATE**

May 07, 2008

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2018

METHOD MODIFICATION RECORD

NONE

SUMMARY OF MODIFICATION

NONE

Under this AOAC® *Performance Tested*SM License Number, 050801 this
method is distributed by:
NONEUnder this AOAC® *Performance Tested*SM License Number, 050801 this
method is distributed as:
NONE**PRINCIPLE OF THE METHOD (1)**

The TEMPO EB test consists of a vial of culture medium and a card, which are specific to this test. The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred from the TEMPO Filler into the card containing 48 wells of three different volumes. The card contains 3 sets of 16 wells (small medium and large wells) with a one-log difference in volume for each set of wells. The card is designed to simulate the Most Probable Number (MPN) method (Cochran 1950)³ (Woodward, 1957)⁴. The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling. The culture medium contains fluorescent pH indicator which, when its pH is neutral, emits a signal detected by the TEMPO Reader. The enterobacteria present in the card assimilate the nutrients in the culture medium during incubation, resulting in a decrease in pH and the extinction of the fluorescent signal. After incubation, the cards are transferred to an automated card reader that detects the fluorescent signal.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of the Internal and Independent method comparison studies are summarized in table 10.

Table 1: Results for inclusivity comparing the TEMPO EB method and the VRBA solid media (1)

#	Species	Strain	Origin	TEMPO cfu per mL*	VRBG cfu per mL*
1	<i>Buttiauxiela agrestis</i>	ADRIA 115	Vegetables	19 / 22	2 / 1
2	<i>Citrobacter diversus</i>	ADRIA 38	unknown	60 / 37	6 / 13
3	<i>Citrobacter diversus</i>	CIP 8294	Collection	26 / 19	<1 / <1
4	<i>Citrobacter freundii</i>	ADRIA 53	Vegetables	21 / 12	8 / 8
5	<i>Citrobacter freundii</i>	CIP 5732	Collection	41 / 37	12 / 5
6	<i>Edwardsiella tarda</i>	CIP 7861	Collection	21 / 34	15 / 19
7	<i>Enterobacter agglomerans</i>	ADRIA 11	Cheese	24 / 16	13 / 8
8	<i>Enterobacter amnigenus</i>	ADRIA 52	Vegetables	2 / 3	4 / 4
9	<i>Enterobacter cloacae</i>	ADRIA 10	Raw milk	17 / 14	13 / 10
10	<i>Enterobacter sakazakii</i>	ADRIA D7	Poultry	91 / 110	58 / 46
11	<i>Escherichia coli</i>	CIP 54117	Collection	12 / 37	9 / 8
12	<i>Escherichia hermanii</i>	ADRIA 395	Meat	6 / 9	24 / 22
13	<i>Escherichia vulneris</i>	ADRIA 127	Raw milk	37 / 31	14 / 10
14	<i>Ewingella americana</i>	ADRIA 71	Vegetables	47 / 37	7 / 3
15	<i>Hafnia alvei</i>	ADRIA 168	Meat	16 / 19	11 / 14
16	<i>Klebsiella oxytoca</i>	CIP 7932	Collection	18 / 16	14 / 14
17	<i>Klebsiella pneumoniae</i>	CIP 8291	Collection	20 / 14	6 / 6
18	<i>Kluyvera ascorbota</i>	CIP 8295	Collection	34 / 15	36 / 45
19	<i>Morganella morganii</i>	CIP A236	Collection	78 / 41	40 / 35
20	<i>Proteus mirabilis</i>	ADRIA 54	Meat	53 / 34	9 / 6
21	<i>Proteus vulgaris</i>	ADRIA 43	Porc	11 / 8	6 / 6
22	<i>Providencia rettgeri</i>	ADRIA 112	Eggs product	39 / 41	36 / 31
23	<i>Providencia stuartii</i>	ADRIA 46	Poultry	34 / 53	15 / 37
24	<i>Salmonella enteritidis</i>	CIP 8297	Collection	78 / 120	95 / 105
25	<i>Salmonella typhimurium</i>	CIP 5858	Collection	60 / 91	57 / 26
26	<i>Serratia liquefaciens</i>	ADRIA 5	Eggs product	210 / 150	86 / 86
27	<i>Shigella flexneri</i>	CIP 8248	Collection	47 / 40	31 / 28
28	<i>Shigella sonnei</i>	CIP 8249	Collection	120 / 40	32 / 27
29	<i>Yersinia enterocolitica</i>	CIP 8027	Collection	55 / 110	6 / 8
30	<i>Pectobacterium(Erwinia) carotovora</i>	CIP 8283	Vegetables	34 / 15	<1 / <1

ATCC: American Type Culture Collection

CIP: Pasteur Institut Collection

ADRIA: French laboratory (Association pour le Développement, la Recherche et l'Innovation Alimentaire)

Bmx: bioMérieux collection

*duplicate test results

Table 2: Results for exclusivity. (1)

#	Species	Strains	Origin	TEMPO cfu/mL	VRBA cfu/mL
1	<i>Acinetobacter spp.</i>	ADRIA 46-2	Poultry	<1	<1
2	<i>Aerococcus vividens</i>	ADRIA 184	Eggs product	<1	<1
3	<i>Aeromonas hydrophila</i>	CIP 5750	Collection	<1	<1
4	<i>Aeromonas sobria</i>	CIP 7433	Collection	<1	<1
5	<i>Bacillus circulans</i>	ATCC 4513	Collection	<1	<1
6	<i>Burkholderia cepacia</i>	Bmx 0504187	Pork	<1	<1
7	<i>Enterococcus durans</i>	ADRIA 181	Eggs product	<1	<1
8	<i>Enterococcus faecalis</i>	ATCC 29212	Collection	<1	<1
9	<i>Flavobacterium indologenes</i>	ADRIA 26	Eggs product	<1	<1
10	<i>Joselia denitrificans</i>	ADRIA 55134	Beef	<1	<1
11	<i>Kurthia zopfii</i>	ADRIA 7621	Unknown	<1	<1
12	<i>Lactobacillus lactis</i>	ATCC 11454	Collection	<1	<1
13	<i>Lactobacillus plantarum</i>	CIP A159	Collection	<1	<1
14	<i>Moraxella spp.</i>	ADRIA 51	Poultry	<1	<1
15	<i>Photobacterium phosphoreum</i>	ADRIA	Fish	<1	<1
16	<i>Pseudomonas aeruginosa</i>	CIP A22	Collection	<1	<1
17	<i>Pseudomonas fluorescens</i>	CIP 5690	Collection	<1	<1
18	<i>Pseudomonas putida</i>	ADRIA 8	Collection	<1	<1
19	<i>Staphylococcus aureus</i>	CIP 658	Collection	<1	<1
20	<i>Staphylococcus epidermidis</i>	ADRIA 150	Raw beef	<1	<1

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Table 10. Summary of Internal and Independent method comparison studies (1)

Food		Lot or Inoculation Level	Log CFU/g ^c TEMPO result	Log CFU/g ^c Reference Result	Comparative Repeatability			t-test p value ¹
					TEMPO ^d	Reference ^d	Method with better repeatability	
Frozen ground beef	Naturally Contaminated	Lot 1	1.11	1.47	0.19	0.42	No difference	0.188
		Lot 2	2.27	2.23	0.15	0.21	No difference	0.785
		Lot 3	2.15	2.33	0.23	0.14	No difference	0.060
Raw ground beef ^a	Naturally Contaminated	Lot 1	2.00	2.15	0.47	0.53	No difference	0.078
		Lot 2	4.17	4.12	0.17	0.17	No difference	0.771
		Lot 3	1.78	1.50	0.51	0.42	No difference	0.156
Raw ground pork	Naturally Contaminated	Lot 1	2.92	3.62	0.47	0.38	No difference	0.012
		Lot 2	3.41	3.79	0.50	0.23	No difference	0.053
		Lot 3	2.50	2.60	0.35	0.24	No difference	0.256
Cooked Roast Beef	<i>Enterobacter cloacae</i> ATCC 700323	Low	1.64	1.76	0.20	0.12	No difference	0.178
		Medium	2.73	2.66	0.15	0.05	Reference	0.271
		High	3.78	3.77	0.22	0.02	Reference	0.840
Fresh Ground Chicken	Naturally Contaminated	Lot 1	4.57	4.66	0.31	0.16	No difference	0.239
		Lot 2	2.68	2.84	0.16	0.08	No difference	0.150
		Lot 3	3.57	3.97	0.27	0.18	No difference	0.004
Frozen Cooked Chicken	<i>Escherichia coli</i> BMX 104116	Low	1.84	1.74	0.14	0.07	No difference	0.249
		Medium	2.80	2.69	0.12	0.02	Reference	0.117
		High	3.84	3.72	0.08	0.05	No difference	0.065
Frozen Cooked Chicken ^a	<i>Enterobacter sakazakii</i> ATCC 51329	Low	2.11	2.09	0.30	0.12	No difference	0.908
		Medium	3.97	4.11	0.10	0.09	No difference	0.116
		High	4.88	4.98	0.21	0.22	No difference	0.461
Grilled chicken	<i>Enterobacter aerogenes</i> ATCC 13048	Low	2.21	2.30	0.19	0.08	No difference	0.377
		Medium	2.75	2.77	0.22	0.07	Reference	0.816
		High	3.85	3.74	0.12	0.08	No difference	0.203

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Heat Processed Fish	<i>Klebsiella oxytoca</i> ATCC 700324	Low	1.90	1.70	0.08	0.15	No difference	0.023
		Medium	2.61	2.67	0.12	0.05	No difference	0.240
		High	3.79	3.67	0.16	0.10	No difference	0.252
Raw Cod	Naturally Contaminated	Lot 1	2.63	3.25	0.25	0.05	Reference	0.004
		Lot 2	1.84	2.26	0.23	0.34	No difference	0.210
		Lot 3	1.24	1.42	0.16	0.04	Reference	0.178
Raw Cod ^{ab}	Naturally Contaminated	Lot 1	3.02	3.27	0.64	0.53	No difference	0.085
		Lot 2	5.43	5.91	0.33	0.33	No difference	0.091
		Lot 3	3.80	3.65	0.38	0.18	No difference	0.461
Frozen Fresh Catfish	Naturally Contaminated	Lot 1	4.54	4.73	0.17	0.11	No difference	0.024
		Lot 2	4.35	4.41	0.27	0.17	No difference	0.385
		Lot 3	4.53	4.55	0.13	0.09	No difference	0.855
Frozen Green Beans	<i>Escherichia coli</i> ATCC 35218	Low	2.14	1.92	0.07	0.12	No difference	0.037
		Medium	3.16	2.90	0.28	0.05	Reference	0.126
		High	3.99	3.85	0.12	0.06	No difference	0.100
Frozen Green Beans ^{ab}	<i>Escherichia coli</i> ATCC 8677	Low	1.99	1.83	0.13	0.24	No difference	0.178
		Medium	2.58	2.45	0.15	0.12	No difference	0.191
		High	4.27	4.26	0.20	0.03	Reference	0.899
Bagged Lettuce	Naturally Contaminated	Lot 1	7.27	7.80	0.25	0.20	No difference	0.009
		Lot 2	6.94	6.91	0.46	0.33	No difference	0.746
		Lot 3	5.61	5.99	0.43	0.16	No difference	0.156
Dry Milk Powder	<i>Citrobacter freundii</i> BMX 100627	Low	1.79	1.72	0.11	0.32	No difference	0.665
		Medium	3.40	3.24	0.11	0.05	No difference	0.024
		High	4.46	4.15	0.18	0.12	No difference	0.051

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Vanilla Ice Cream	<i>Escherichia coli</i> BMX 100454	Low Medium High	1.80 2.84 4.00	1.62 2.59 3.75	0.20 0.13 0.20	0.20 0.46 0.09	No difference Tempo No difference	0.040 0.243 0.086
Pasteurized Whole Milk	Naturally Contaminated	Lot 1 Lot 2 Lot 3	4.78 4.41 4.34	4.88 4.29 4.30	0.18 0.07 0.17	0.07 0.17 0.36	No difference No difference No difference	0.367 0.148 0.852
Hash brown Potatoes	<i>Enterobacter cloacae</i> BMX 102271	Low Medium High	2.95 4.46 5.59	2.82 4.42 5.38	0.15 0.09 0.14	0.04 0.03 0.02	Reference No difference Reference	0.149 0.394 0.035
Eggs	<i>Salmonella enterica ssp. arizonae</i> BMX 109249	Low Medium High	2.36 3.45 4.33	2.35 3.30 4.36	0.07 0.13 0.15	0.07 0.08 0.03	No difference No difference Reference	0.904 0.086 0.620
Rice	<i>Enterobacter cloacae</i> BMX 100057	Low Medium High	1.94 2.70 3.67	2.03 2.68 3.65	0.06 0.05 0.30	0.07 0.08 0.05	No difference No difference Reference	0.185 0.717 0.868
Dry Pet Food	<i>Klebsiella pneumoniae</i> ATCC 700603	Low Medium High	1.92 2.64 3.80	2.03 2.62 3.46	0.20 0.07 0.07	0.08 0.04 0.07	No difference No difference No difference	0.191 0.494 0.220

¹ **BOLD** numbers indicate significance (p-value of ≤ 0.05) at the 95% confidence level

^a Independent laboratory testing

^b Independent laboratory testing shared matrix

^c Standard deviation

¹ **BOLD** numbers indicate significance (p-value of ≤ 0.05) at the 95% confidence level

^a Independent laboratory testing

^b Independent laboratory testing shared matrix

^c mean log count for replicate tests

^d Standard deviation for the replicate log counts

REFERENCES CITED

1. Johnson, Ron, Mills, John, and Devulder, Grégory., Evaluation of the TMEPO® *Enterobacteriaceae* assay method in a Variety of Foods, AOAC® *Performance Tested*SM certification number 050801.
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4. Woodward, R. L., How probable is the most probable number? (1957) *J. Am. Water Works Assoc.*49:1060-106